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Research Article

Detailed analysis of prey taxonomic composition indicates feeding habitat partitioning amongst co-occurring invasive gobies and native European perch

Joanna Grabowska[1], Mateusz Płóciennik[2], Michał Grabowski[2]

1 *University of Lodz, Faculty of Biology and Environmental Protection, Department of Ecology and Vertebrate Zoology, Banacha 12/16, 90-237 Lodz, Poland*
2 *University of Lodz, Faculty of Biology and Environmental Protection, Department of Invertebrate Zoology and Hydrobiology, Banacha 12/16, 90-237 Lodz, Poland*
Corresponding author: Joanna Grabowska (joanna.grabowska@biol.uni.lodz.pl)

Abstract

One of the negative impacts of non-native invasive species on trophic interactions in an invaded ecosystem occurs via increased interspecific competition for food resources between the invader and local species of the same food niche. In freshwaters, there are usually several fish species that feed on similar food resources. Ponto-Caspian gobies are amongst the most successful and widespread invaders colonising European waterways. They have a wide food niche and an opportunistic feeding strategy, with a focus on benthic invertebrates and piscivory occurring occasionally mainly in the case of large individuals. Competition with native percids for food resources is predicted on the basis of high dietary overlap. However, studies published so far provide no unequivocal answer. In order to resolve this question, we conducted a comparative taxonomic analysis of gut content, with an emphasis on chironomids and amphipods, of the invasive monkey goby (*Neogobius fluviatilis*), racer goby (*Babka gymnotrachelus*) and the native Eurasian perch (*Perca fluviatilis*) occurring sympatrically in a large lowland European river, the Bug River in Poland. We found that each species forages in slightly different habitats, as indicated by the different composition of prey species in the gut content. This suggests feeding niche partitioning between the studied species facilitating their co-existence and reduction or avoidance of competition for food resources. Resource partitioning regarding prey types and foraging habitats is a mechanism for permitting the co-existence of closely-related alien gobies with similar food preferences in the invaded waters and co-occurrence with local species. This mechanism can contribute to their invasion success, as observed in European waters during the recent decades. We also demonstrate that precise prey identification to the lowest possible taxon is crucial to reveal the dietary overlap between co-occurring fish species and to predict the impact of alien invaders on native species through interspecific competition, as well as to recommend such an approach in studies upon fish foraging strategies.

Key words: Feeding niche, fish diet, non-native species, resource partitioning, trophic interactions

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Introduction

Freshwater ecosystems, together with their biodiversity, are amongst the most threatened and altered environments on the planet, due to the intensive human exploitation of water resources. Widespread invasions of introduced non-native species are amongst the five main threats for such ecosystems (Dudgeon et al. 2006). Successful biological invasion depends on several factors, including interactions of

the newcomer with the local biota that can be particularly critical for the further fate of an alien species. The arrival and establishment of non-native fish species leads to a number of changes in the ecosystem, particularly in the pre-existing food web. The ecological consequences of such interference depend on the trophic position of the invader and the abundance of species that belong to the same ecological guild in the recipient ecosystem, as well as the availability of resources they share. Piscivorous invaders have a high potential for harmful effects on the ecosystem, especially if native predators are rare or absent (Howeth et al. 2016). The other frequent assumption in fish invasion ecology is that negative impacts of invasions on trophic interactions occur via increased inter-specific competition for food resources (see, for example, Gozlan at al. (2010); Cucherousset and Olden (2011)). In freshwaters, there are usually several fish species that feed on similar food resources. Their co-existence is made possible by resource partitioning, such as different activity patterns or different use of space and food resources. Resource partition is an effective way of reducing competition and it applies also in the case of alien species introductions to recipient fish assemblages (Britton et al. 2010; Tran et al. 2015). Comparative studies on diet of functionally similar fish species in sympatry require detailed prey identification to conclude about resource partitioning or diet overlap (Dukowska et al. 2013; Lik et al. 2017). The identification of prey to the lowest possible taxon has potential value for determining the habitat preferences of both a prey and, based on that, its predator, as for fish, feeding habits and habitat preferences are often interconnected.

As fish species change their trophic status over the course of their lives, displaying ontogenetic niche shifts, many European freshwater fish do not fall into discrete trophic categories (Noble et al. 2007; Specziár and Rezsu 2009), but should rather be classified into collective groups, for example, insectivore/piscivore (Noble et al. 2007) or, according to other classifications, zoobenthivorous/piscivorous or zooplanktivorous/zoobenthivorous/piscivorous (Aarts and Nienhuis 2003). Macrozoobenthos is an important food for many species. The classification of fish that occur in the Rhine and Meuse rivers into ecological guilds showed that 49 out of 56 species included in the study had a zoobenthivorous phase in their life and they comprised ca. 40% of all species of fish there. A similar pattern can be found in other rivers belonging to the Central European biogeographical region (*sensu* Reyjol et al. (2007)). The region was distinguished by the composition of ichthyofauna and encompasses watersheds from the River Elbe in the west, through the Rivers Oder, Vistula, Neman to Narva in the east, as well as the Swedish and Finnish Baltic river systems. In recent decades, the rivers in this region have faced rapid invasion by five Ponto-Caspian goby species (Grabowska et al. 2008; Rakauskas et al. 2018; Kvach et al. 2021). The contribution of invasive gobies to local fish assemblages varies between watersheds and changes over time (Polačik et al. 2009; Borcherding et al. 2011; Janáč et al. 2018; Gaye-Siessegger et al. 2022). They are considered to have a wide food niche and an opportunistic feeding strategy. The diet differs between goby species, but benthic invertebrates, in particular amphipods and chironomid larvae, are their main prey, while piscivory occurs only in the case of larger individuals of some species (see review by Grabowska et al. (2023)). Their strong competition with native fish species was expected on the basis of high diet overlap, especially with native percids (Copp et al. 2008; Adámek et al. 2010; Kocovsky et al. 2011; Borcherding et al. 2019).

The racer goby *Babka gymnotrachelus* (Kessler, 1857) and monkey goby *Neogobius fluviatilis* (Pallas, 1814) are the first two invasive gobies that arrived in the Vistula River system, almost at the same time, in the mid-1990s and soon spread there rapidly (Grabowska et al. 2008). They were recorded for the first time in the Baltic Sea Basin in the Bug River, right tributary of the Vistula, being part of the central invasion corridor for the Ponto-Caspian aquatic fauna from the Black Sea Basin (Semenchenko et al. 2011). Until 2008, they were the only goby species present in the Vistula River system (Grabowska et al. 2008). The frequency of occurrence of racer and monkey goby in the Bug River (main right tributary of the Vistula) in 2007–2009 was 32% and 68%, respectively (Penczak et al. 2010), reaching even as much as 85% and 100%, if we consider only the lower section of that river, where their first expansion occurred. At all sites, alien gobies co-occurred with Eurasian (European) perch *Perca fluviatilis* Linnaeus, 1758, which was recorded in 96% of the 56 sites surveyed along 587 km of the river (Penczak et al. 2010).

The diet of racer and monkey gobies is similar and mainly comprises benthic macroinvertebrates, though chironomid larvae, other insects larvae, amphipods, molluscs and occasionally also small fish fry, predominate in their diet (Grabowska and Grabowski 2005; Kakareko et al. 2005; Grabowska et al. 2009; Didenko et al. 2017, 2021a, b, 2022a). It can be expected that these two alien gobies share food resources with native perch that feed on similar prey, at least during some stages of their ontogeny (Kornijów 1997; Rezsu and Specziár 2006; Kornijów et al. 2016). Considering that, at the time of sampling for our study, racer goby and monkey goby had already established abundant populations in the Bug River and had co-occurred there with European perch for around 10–15 years since their first arrival, we aimed to determine whether such co-existence is based on resource partitioning.

We hypothesised that the three fish species, although potentially feeding on the same type of prey, slightly vary the composition of their diet, for example, by exploring different foraging habitats to minimise interspecific competition when co-occurring in the same section of a river. We verified this prediction by comparative analysis of fish diet, based on the detailed taxonomic identification of selected prey taxa, focusing on chironomids and amphipods, which can differ in terms of the occupied microhabitats. We achieved this by analysing the gut content of racer goby, monkey goby and European perch occurring sympatrically in a large lowland river flowing through the East European Plain, which constitutes a crucial part of the Central Invasion Corridor for westward expansion of the Ponto-Caspian fauna, as defined by Bij de Vaate et al. (2002).

Materials and methods

Fish sampling and site description

All the three studied species, monkey goby, racer goby and perch, were sampled from three sites (Fig. 1) located in the Bug River (the Vistula River system's largest eastern tributary, Baltic Basin, Poland) in 11–14 August 2007. The Bug River maintained its natural character of a lowland, meandering river within a wide valley. It is 772 km long and the watershed covers 39,420 km^2. Its sources are in Ukraine, but after 185 km, a stretch of 363 km comprises the border be-

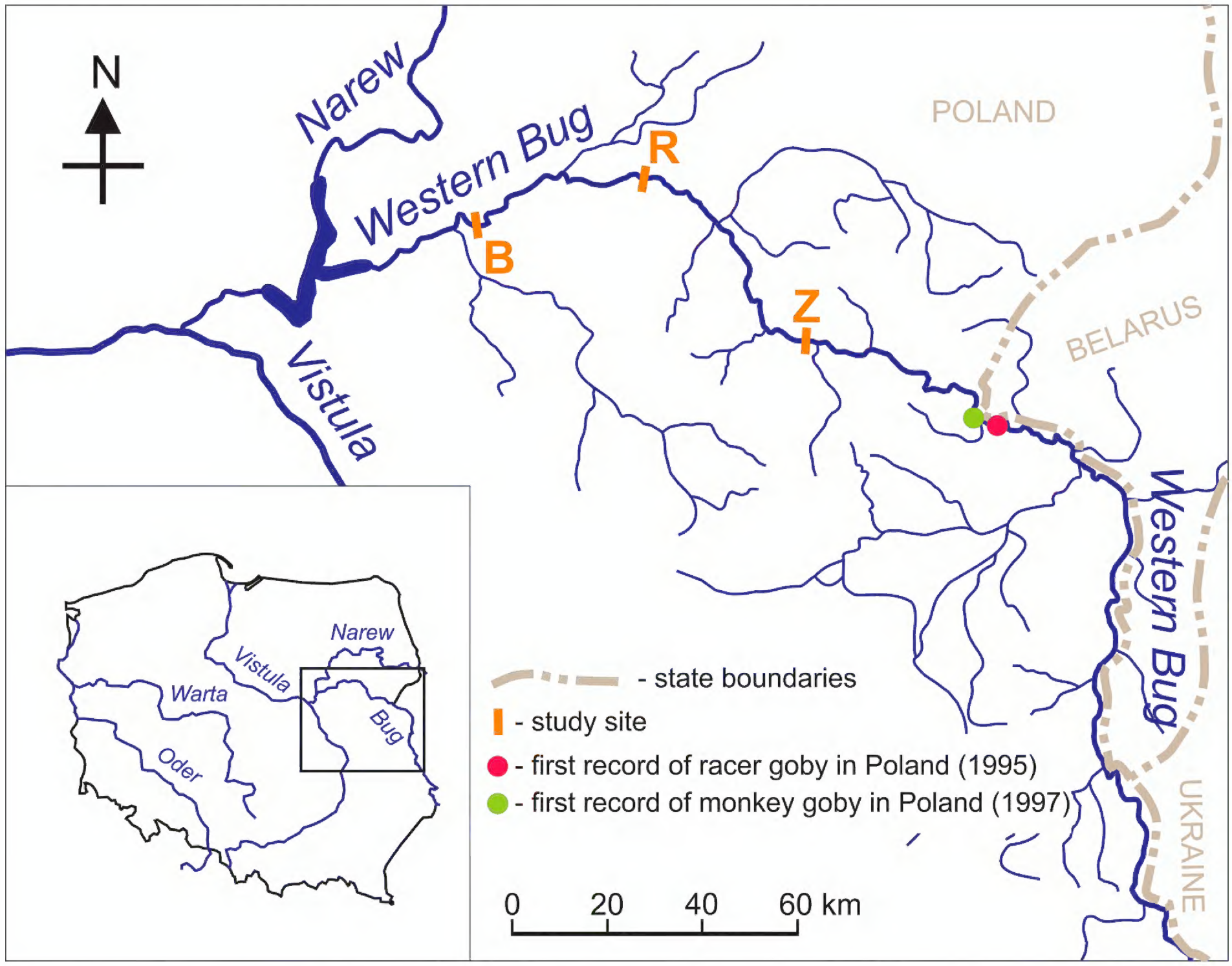

Figure 1. Location of sampling sites Z, R, B in the Western Bug River and places of first record of racer goby (red dot) and monkey goby (green dot) in Poland.

tween Poland, Ukraine and Belarus. Later, it turns to the west until it joins the Narew River, shortly before its confluence with the Vistula River. Sampling sites Z (52°23.57333'N, 22°42.25833'E), R (52°42.08667'N, 22°09.73333'E) and site B (52°37.41000'N, 21°35.03000'E) were situated in the lower Bug River, which flows entirely through the territory of Poland. The river in this section is more than 100 m wide and relatively shallow, but with a heterogeneous depth profile (pools and riffles), mainly with a sandy bottom, though with some contribution of gravel and stones (Table 1) and scattered submerged vegetation, such as *Elodea canadensis*, *Potamogeton perfoliatus*, *Myriophyllum* sp. The riverbed is naturally meandering with a sequence of eroded and deposited banks with some emerging macrophytes (*Typha* sp., *Scirpus* sp., *Juncus* sp., *Sparganium* sp., *Glyceria maxima*). Only in site B, there was a short section of the bank that had a limestone embankment and a paved area at a small bay and a platform created as a recreational area in the village. The racer goby was very abundant there, as it used such artificial structures as hiding places. The surrounding landscape mainly comprised pastures and other agricultural lands (Table 1).

Fish were sampled at depths from 0.5 m to 1.7 m, along the riverbank by electrofishing with a battery-powered unit, 350 V, 20–100 Hz, wading ca.100 m upstream along the bank and from the boat drifting 500 m downstream.

Table 1. Morphometry of sampling sites: Z, R, B in the Western Bug River. Explanations: a) – absent, + very little/few, ++ common, +++ abundant; b) % of bed cover; c) % of bank overgrown; d) pa- pastures, ł- meadows, cr- cropland, bl- buildings.

	Site	Z	R	B
1.	Distance from the mouth [km]	176.7	108.2	46.0
2.	Mean width [m]	120	109	114
3.	Mean depth in current [m]	1.8	0.8	1.5
	Maximal depth in the current [m]	> 3.0	1.5	> 3.0
4.[a)]	Pools/riffles	+++	+	+
5.	Mud cover 0–100 [%]	0	5	0
	Bottom substrate: sand/gravel/stone [%]	70/20/10	50/30/20	60/20/20
6.[b)]	Submerged plants	10	20	10
7.[c)]	Emerged plants	30	50	30
8.[a)]	Trees along banks	+++	+++	+
9.[d)]	Adjacent area	pa, ł	cr, pa, bl	bl, ł, pa
10.	Water temperature [°C]	19.0	19.1	19.2
11.	pH	8.62	8.75	8.89
12.	Dissolved oxygen [mg O^2 dm^{-3}]	13.4	5.9	9.26
13	Conductivity [µS cm^{-1}]	509	502	476

Fish diet analysis

Fish were anaesthetised (MS-222) and preserved in 4% formaldehyde. In the laboratory, the fish were measured for total length (L_T; to the nearest 1 mm) and weighed (with 0.01 g accuracy). Their alimentary tracts were dissected. The gut contents (in each fish, the same section of alimentary tract, i.e. stomach and first half of intestine) were weighed (to 0.0001 g accuracy) and prey items were identified under a stereomicroscope. Chironomidae larval stages can be identified to genera or groups of closely-related species, but only rarely to the species level. Their remains from the fish gut lack many features that are necessary for precise identification and, thus, following the main key used in this study (Brooks et al. 2007), they were identified to the morphotype cf. level.

Animal prey remains were identified to the lowest readily recognisable taxon, counted and the proportional weight was estimated. The percentage contribution by weight of each food category to the biomass of total stomach content was estimated visually (Hyslop 1980) and then recalculated into real weights, based on the weight of total gut content. The frequency of occurrence (defined as percentage of fish guts containing given prey category in relation to the total number of fish with guts containing any food: %F), percentage of biomass (weight of given food category in relation to total weight of gut content: %B) and relative abundance (number of given prey category in relation to total number of prey: %N) were quantified for each food category at each sampling site.

The Amundsen et al. (1996) modification of the Costello (1990) graphical method was applied to describe feeding strategy and to identify dominant prey items for the fish species, as well as feeding phenotypic plasticity.

Dietary overlap between each pair of fish species was calculated using Schoener's index (Wallace 1981): $\alpha = 1–0.5\ [\Sigma_{n=1}\ (p_{ij} - p_{ik})]$, where p_{ij} is the proportion of the *i*th resource used by species *j* and p_{ik} is the proportion of the *i*th resources used by

species *k*; overlap values exceeding 0.6 were regarded as high or biologically significant (Wallace 1981). As the proportion in Schoener's index calculation, we used %N proportion: numbers of given prey type to the total number of prey found in fish gut. To show how the accuracy of prey identification influences the evaluation of dietary overlap, we estimated it based on protocol 1 – considering main food categories, i.e. prey pooled into taxonomic groups usually applied in fish diet studies, for example, Amphipoda, Chironomidae (called later Schoener's index 1) and protocol 2 – considering detailed food categories, i.e. prey identified to the lowest possible taxonomic level (called later Schoener's index 2).

To compare the taxonomic composition of the diet between fish species overall (all sites pooled) and at each sampling site, one-way permutation analysis of similarity (ANOSIM, Bray-Curtis similarity coefficient) was used, based on prey. ANOSIM is analogous to an ANOVA procedure, with non-parametric permutation applied to rank similarity matrix of samples. The similarity percentage procedure (SIMPER) was used to identify which prey taxa were most likely responsible for the patterns detected by ANOSIM. SIMPER provided the average dissimilarities between the species and identified which prey taxa made the greatest contribution to any dissimilarities between analysed categories (Clarke and Warwick 1994). All multivariate analyses were performed using PAST software (ver. 3.15; Hammer et al. (2001)).

Dietary niche width was calculated as a Simpson diversity index: $1 - D = 1 - \Sigma pi^2$ and Shannon diversity index: $H = -\Sigma pi \log_2 pi$, where *pi* is the proportion of different prey in the diet (Ghent 1991).

Results

The fish species recorded from the sampling sites were mainly bleak *Alburnus alburnus* and roach *Rutilus rutilus*. These two species constituted 45%–64% of all fish caught at the study sites and dominated in abundance along the whole middle and lower river course. The other species that occurred at all three sites were common bream *Abramis brama*, white bream *Blicca bjoerkna*, pike *Esox lucius*, chub *Squalius cephalus*, ide *Leuciscus idus*, common rudd *Scardinus erythrophtalmus*, spined loach *Cobitis taenia* and bitterling *Rhodeus sericeus* (Suppl. material 1). The contribution of studied fish species to the fish assemblages at studied sites Z, R, B was as follows: racer goby (2.8%, 1.6%, 4.3%), monkey goby (0.7%, 4.0%, 6.0%) and perch (7.3%, 6.4%, 5.2%), respectively. However, for further analysis we selected perch species of size range similar to gobies, i.e. almost all large perch (> 150 mm) were excluded in diet analysis and the majority of individuals were juveniles, i.e. in benthivorous stage of ontogeny.

In total, 63 individuals of racer goby, 77 of monkey goby and 62 of perch were caught in three sampling sites. In four out of 202 dissected individuals, the alimentary tracts were empty and not considered in further analysis.

In all three fish species, prey belonging to Amphipoda, Chironomidae larvae and pupae, Gastropoda, Trichoptera larvae, Coleoptera larvae, Oligochaeta and Hirudinea were found in the diet (Suppl. material 2). Odonata larvae and Pisces were not recorded from any monkey goby and Bivalvia were not eaten by perch. Altogether, we distinguished 11 main prey categories and, additionally, four accountable categories of fish gut content for which only biomass was estimated, the latter being Mollusca not identified, detritus, sand and fish eggs. The contribution

Table 2. Diet composition of the European perch, racer goby and monkey goby (mean, minimum and maximum total length of fish – TL) expressed as relative abundance (%N) and relative biomass (%B) of main food categories in gut content at the three studied sites (Z, R, B).

Species	Racer goby						Monkey goby						European perch			
Site	Z		R		B		Z		R		B		Z		R	
N of specimens	34		20		27		20		32		24		31		30	
Mean TL [mm]	70.24 (±14.50)		60.70 (±15.53)		74.65 (±14.50)		96.90 (±27.36)		92.36 (±14.68)		85.86 (±16.03)		95.24 (±29.95)		98.154 (±7.95)	
Min-max TL [mm]	51–101		42–96		52–103		52–124		61–120		54–112		53–150		84–115	
Food categories	%N	%B	%N	%B	%N	%B	%N	%B	%N	%B	%N	%B	%N	%B	%N	%B
Amphipoda	27.5	48	19.6	65	13.8	38	16.7	22	6.1	19			54.9	53	54.2	57
Chironomidae larvae	43.7	12	67.4	29	78.8	17	39.7	8	76.2	44	76.1	17	20.4	6	23.8	5
Chironomidae pupas	4.8	4	4.3	1			3.8	3	5.4	7	1.0	2	2.6	2	7.0	3
Trichoptera larvae	0.6	> 1					12.8	4	2.2	3	18.1	18	0.4	> 1	0.5	2
Odonata larvae	0.6	> 1							0.5	6	1.0		1.7	> 1		
Coleoptera larvae	1.8	2			2.5	1					1.0	4	0.9	> 1	1.9	2
Bivalvia	14.4	19	2.2	1			11.5	26	7.9	5	1.9	6				
Gastropoda	5.4	4	6.5	4	2.5	10	12.8	27	1.8	2					1.4	2
Mollusca not ident.		6								14						
Oligochaeta					1.3	1					1.0	6			0.9	6
Hirudinea	0.6	> 1					2.6	10								
Pisces	0.6	> 1			1.3	21							19.1	34	10.3	23
Detritus (plant)						12						23		2		
Sand												12				
Fish eggs											12	12				

of each food category varied between sites (Table 2), but Chironomidae larvae were the dominant prey for both goby species, considering both abundance and frequency of occurrence and of a secondary importance for perch (Fig. 2A–C), which fed predominantly on amphipods, which dominated in abundance and biomass of their food (Table 2). These crustaceans were also found in more than 50% of racer goby guts (Fig. 2B), constituted 38–65% of food biomass in that fish species (Table 2) and were subdominant prey, considering their contribution to the total prey abundance (Fig. 2B). Amphipods were less frequently eaten by monkey goby (Fig. 2C) and their contribution to prey abundance and total biomass depended on the site (Table 2).

The plot of prey specific abundance (%Nps) and frequency of occurrence (%F) of the main components of the diet showed that chironomid larvae were the prey of higher importance for gobies, while, for European perch, amphipods were more important (Fig. 2). The prey of high importance means that it has been eaten by more than half the individuals and have high contribution in specific abundance. Considering feeding strategy, both gobiid species and European perch are generalist feeders, relying on several prey taxa with a relatively low prey-specific abundance, being mainly located in the lower part of the diagram.

Diet overlap, as calculated for the 11 main food categories (Schoener's index 1), occurred amongst all three species if data for all sites were pooled, which indicated that their prey spectrum was very similar (Table 3). If analysed separately for each site, the dietary overlap was very high (ca. 0.8) only between the gobies at all three

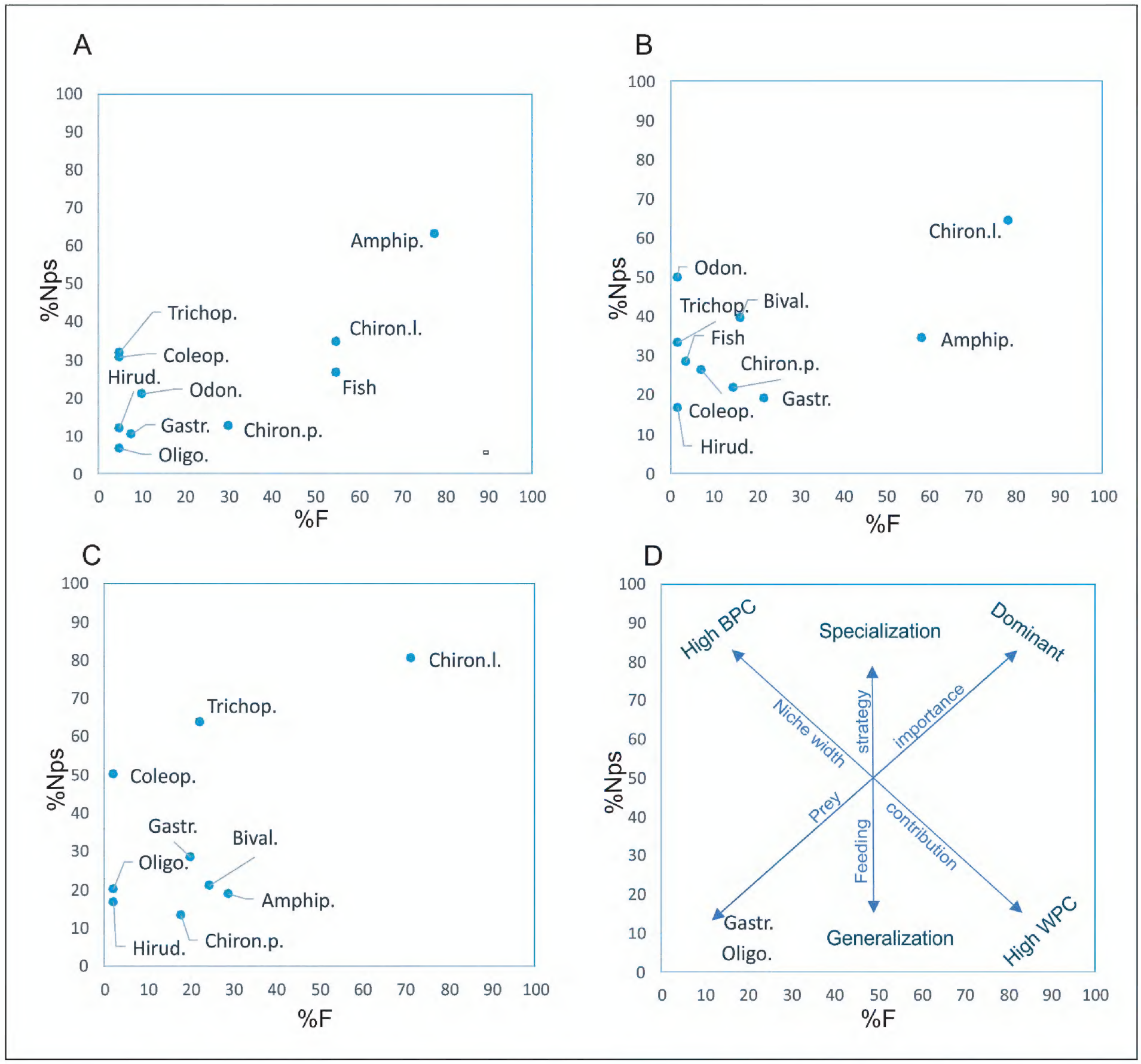

Figure 2. Feeding strategy displayed using the Amundsen et al. (1996) modification of the Costello (1990) graphical method for **A** European perch **B** racer goby **C** monkey goby, in the Bug River (only main food categories included and data from sites pooled for species) **D** explanatory diagram (%Nps – prey specific abundance; %F – frequency of occurrence defined as percentage of fish guts containing given prey category in relation to the total number of fish with guts containing any food).

sites, moderate (0.5) between racer goby and perch, while there was no dietary overlap between monkey goby and perch at any site (Table 3).

Up to 56 taxa (including 28 chironomids and 4 amphipods) dominated the food categories shared by the three studied fish species. For the analysis, we rejected taxa that were found in only one fish, which reduced the number of prey taxa to 42 (including 24 chironomids). The values of Schoener's index 2 indicated that there was no dietary overlap between gobies and perch at any site, but there was also no dietary overlap between racer goby and monkey goby at site B or it was moderate (ca. 0.5) at the other two sites, Z and R (Table 3). ANOSIM similarity analysis showed that the mean abundance of Chironomidae, Amphipoda and other taxa in fish diets varied between fish species, when data from all individuals of each fish species from all sites were pooled, while

Table 3. The dietary overlap estimated, based on two protocols: calculated for general (Schoener's index 1) and detailed food identification (Schoener's index 2) categories. Pairwise comparisons (Bonferroni test) of fish diet following one-way ANOSIM and SIMPER analysis based on detailed identified food categories.

Comparisons	Schoener's index 1	Schoener's index 2	ANOSIM		SIMPER
			R	p	dissimilarity
Fish species (site pooled)					
perch vs racer goby vs. monkey goby			0.1893	0.0001	91.08
perch vs. racer goby	0.826	0.424	0.1333	0.0060	87.82
perch vs. monkey goby	0.721	0.220	0.3520	0.0030	94.95
racer goby vs. monkey goby	0.818	0.480	0.1266	0.0030	91.22
Site Z					
perch vs. racer goby vs. monkey goby			0.2953	0.0001	91.07
perch vs. racer goby	0.530	0.346	0.3543	0.0003	88.47
perch vs. monkey goby	0.400	0.175	0.0353	0.9140	97.26
racer goby vs. monkey goby	0.784	0.572	0.2656	0.0470	92.53
Site R					
perch vs. racer goby vs. monkey goby			0.1883	0.0010	91.47
perch vs. racer goby	0.491	0.274	0.1393	0.0003	84.30
perch vs. monkey goby	0.370	0.175	0.3250	1.0000	94.57
racer goby vs. monkey goby	0.816	0.556	-0.0870	1.0000	90.53
Site B					
racer goby vs. monkey goby	0.790	0.389	0.3243	0.0130	88.83

if analysed between fish species within each site, showed some exceptions, for example, there were no differences between perch and monkey goby at sites Z and R and between racer goby and monkey goby at site R (Table 4). SIMPER identified the taxa that contributed the most to the overall dissimilarity between the diets of the fish species (Table 4).

Ten taxa, i.e. *Glyptotendipes* cf. *pallens*, *Dikerogammarus villosus*, *Chironomus* cf. *riparius*, Pisces, *Pontogammarus robustoides*, *Polypedilum* cf. *nubeculosum*, *Micro-tendipes pedellus*-type, gastropods, caseless larvae (*Hydropsyche* sp.) of Trichoptera and *Rheocricotopus* cf. *chalybeatus*, out of 56 analysed, contributed to 80% of the overall dissimilarity amongst the diets of perch, racer and monkey gobies, though the mean abundance of particular prey varied between sites (Fig. 3).

The perch mainly fed on amphipods, i.e. *P. robustoides* at site Z, *D. villosus* at site R and fish at sites Z and R. Amongst the Chironomidae larvae, the *G.* cf. *pallens* contributed the most to the perch diet at each site. This chironomid was the most abundant in the diet of racer goby at sites Z and B. *D. villosus* was also an important food item of racer goby at site Z. Sphaeridae were not recorded in the diet of perch, but contributed to the diet of both goby species, especially at site Z, where monkey goby fed also on gastropods. Contrary to the other two co-occurring fish species, monkey goby consumed many caseless trichopteran larvae, as well as the chironomids: *C.* cf. *riparius* at all sites and *P.* cf. *nubeculosum* at site B.

The prey diversity was lower for perch than for gobies. Concerning the latter, prey diversity tended to be higher for racer goby than for monkey goby at sites Z and R; however, it was equal at site B (Fig. 4A, B), where the contribution of various chironomid species to the diet of both gobies was very high.

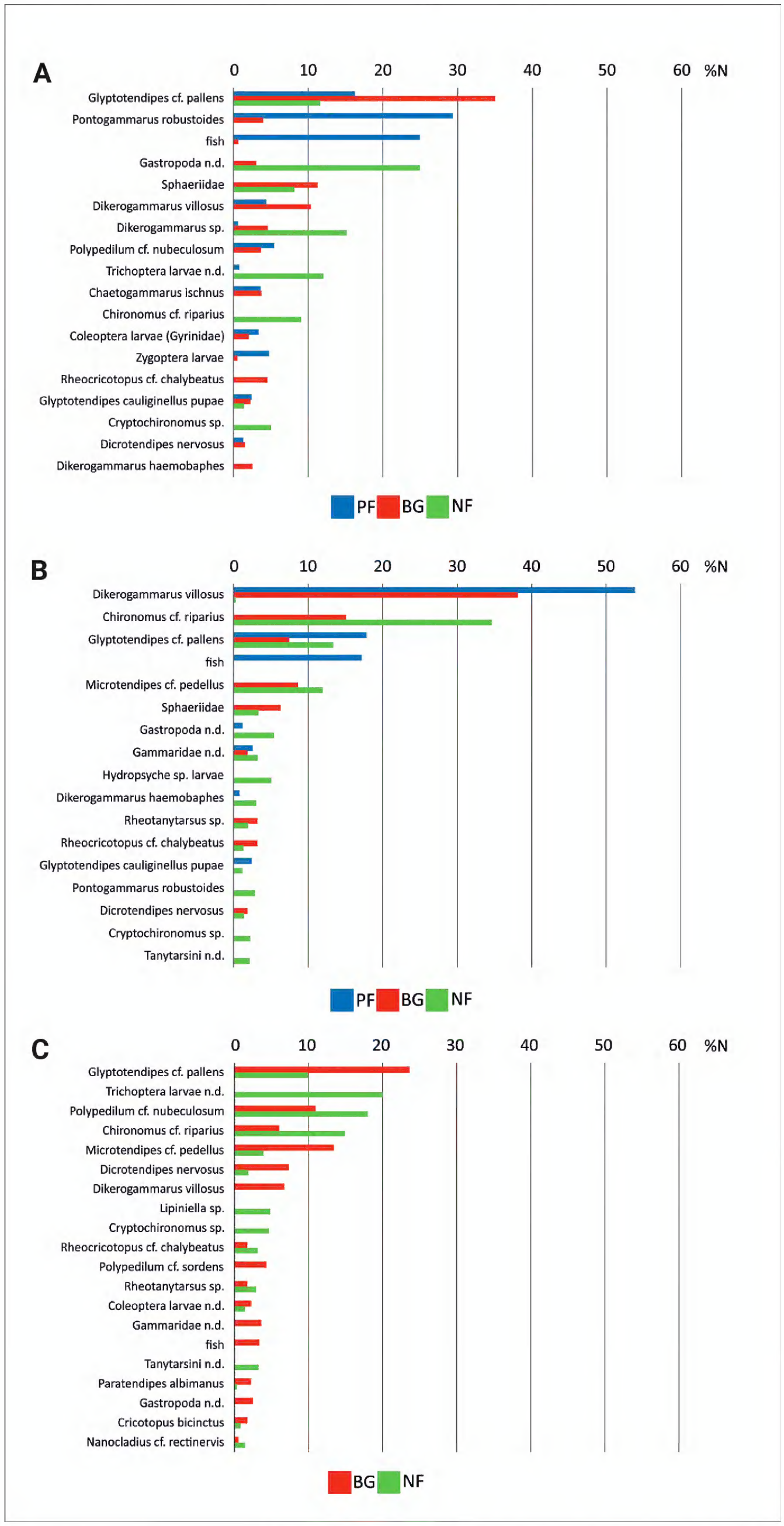

Figure 3. Average relative abundance of prey taxa (%N) in the gut contents of PF – European perch, BG – racer goby, NF – monkey goby, which, in total, contributed to 95% of dissimilarity (SIMPER) amongst fish species diets at **A** site Z **B** site R and **C** site B. n.d. – means not identified.

Table 4. Results of SIMPER analysis identifying prey categories with the highest contribution to the overall dissimilarity amongst fish species diets and their mean relative abundance (%N) in diets of perch (PF), racer goby (BG) and monkey goby (NF).

Taxon	Contribution %	Cumulative %	Mean PF	Mean BG	Mean NF
Glyptotendipes cf. *pallens*	15.1	15.1	17.0	28.0	11.9
Dikerogammarus villosus	14.1	29.2	29.1	13.3	0.1
Chironomus cf. *riparius*	9.6	38.8	0.0	3.8	22.8
Pisces	7.8	46.6	21.0	1.3	0.0
Pontogammarus robustoides	6.4	53.0	14.6	2.3	1.3
Polypedilum cf. *nubeculosum*	4.8	57.8	2.8	5.1	6.1
Sphaeriidae	4.4	62.3	0.0	7.5	3.8
Microtendipes cf. *pedellus*	4.3	66.5	0.0	4.8	6.8
Gastropoda not identified	4.2	70.7	0.6	2.4	8.0
Hydropsyche sp. larvae	3.6	74.3	0.5	0.0	8.9
Dikerogammarus not identified	2.4	76.7	0.3	2.7	3.4
Rheocricotopus cf. *chalybeatus*	2.1	78.8	0.0	3.6	1.6
Gammaridae not identified	1.9	80.7	1.3	2.4	1.5
Dicrotendipes cf. *nervosus*	1.9	82.6	0.6	3.1	1.2
Glyptotendipes cauliginellus pupae	1.6	84.2	2.4	1.3	0.9
Chaetogammarus ischnus	1.5	85.7	1.8	2.2	0.0
Cryptochironomus sp.	1.4	87.1	0.0	0.0	3.6
Dikerogammarus haemobaphes	1.3	88.4	0.4	1.5	1.4
Rheotanytarsus sp.	1.2	89.6	0.0	1.3	1.8
Coleoptera (*Gyrinus* sp.)	1.1	90.7	1.7	1.2	0.0
Lipiniella moderata	1.1	91.8	1.2	0.3	1.5
Tanytarsini not identified	1.1	92.9	0.0	0.3	2.4
Zygoptera larvae	1.0	93.8	2.4	0.3	0.0
Trichoptera larvae not identified	0.9	94.7	0.0	0.0	2.3
Coleoptera larvae not identified	0.7	95.5	0.5	0.9	0.4
Polypedilum cf. *sordens*	0.5	95.9	0.0	1.2	0.0

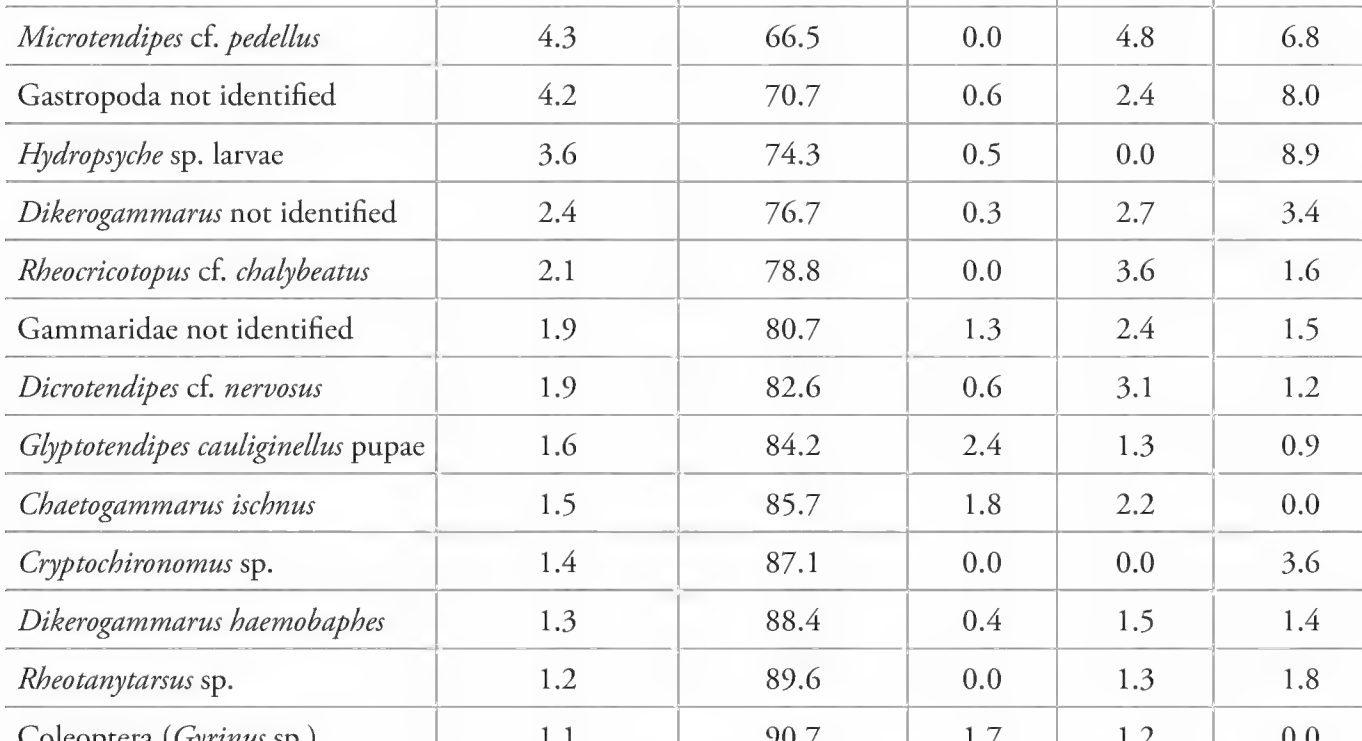

Figure 4. Dietary niche width of European perch, racer goby and monkey goby at three study sites Z, R, B, in the Western Bug River calculated as **A** – Simpson diversity index: 1 – D and **B** – Shannon diversity index: H.

Discussion

The studied fish species, native European perch as well as non-native racer goby and monkey goby, fed on similar prey taxa, which suggests a high dietary overlap. Nevertheless, more detailed identification of taxa in the most abundant food categories, i.e. chironomid larvae and amphipods, revealed that they foraged on different prey at sites where they co-occurred. Thus, although the majority of prey taxa were recorded in guts of all the three studied fish species, their contribution to the diet at a given site was different. This supports the hypothesis of resource partitioning to avoid competition for food between native and non-native species.

Although several experimental studies showed the higher competitive ability of invader *versus* native species and the greater potential of the former to utilise resources (Kakareko et al. 2013; Grabowska et al. 2016; Mofu et al. 2019), there are mechanisms to avoid such antagonistic interactions in natural environments. One of observed functional responses to introduction of non-native species is trophic niche divergence to minimise the trophic interactions between competing species (Tran et al. 2015; Britton et al. 2018). It facilitates the integration of introduced species into food webs (Britton et al. 2018). Contrary to an expected negative impact of gobies on co-occurring native fish species of similar trophic position, there was no clear evidence for that from field surveys (Piria et al. 2016; Ramler and Keckeis 2019). Instead, spatial segregation between species of the same feeding guild was suggested, which was assumed to arise from different prey dominating the diet, for example, racer goby and native ruff and perch in the Vistula River (Grabowska and Grabowski 2005).

Moreover, our findings proved that accuracy in taxonomic identification of prey taxa is essential to provide reliable data for dietary overlap or resource partitioning assessment. It is especially crucial in the case of fish species, for example, racer goby and monkey goby, feeding on the same type of prey that is very diverse considering its body size and occupied microhabitats. Identification of prey to the lowest possible taxon also allows us to determine the habitat preferences of fish species based on the knowledge of their prey microhabitat preferences. Our results showed that, in the case of gobies, resource partitioning is realised by utilisation of different habitats.

Native perch vs. alien gobies

Both goby species and European perch fed on the macrozoobenthos. The perch is known to shift toward piscivory with its ontogenetic development (Hjelm et al. 2000; Rezsu and Specziár 2006). In our study, only a few individuals of perch, i.e. > 120 mm predate on juveniles of fish. Bleak *Alburnus alburnus*, bitterling *Rhodeus amarus* and unidentified fry of other cyprinids were recorded in its diet in the Bug River. In the case of the studied gobies, we recorded piscivory only in the racer goby, but identification of the prey species was impossible due to the advanced stage of the digestion process. Piscivory was already reported, both for racer goby and monkey goby, but such a food category was not considered important and generally occurred only in the largest individuals (Grabowska and Grabowski 2005; Grabowska et al. 2009; Grabowska et al. 2023).

In general, the diets were more similar between the goby species than between either of the gobies and perch. However, the diet of perch was more similar to that of the racer goby than to that of the monkey goby. Both the Eurasian perch and the racer goby fed on prey that indicated their association with macrophytes.

Macrophyte patches are refuges for small fish, as well as hiding places or substrate for several macrozoobenthic groups, such as amphipods, insect larvae, for example, Diptera and Zygoptera larvae or gastropods, that are attractive food for many fish species (Gulati et al. 1990; Van den Berg et al. 1997; Dukowska et al. 2012; Dukowska and Grzybkowska 2014; Grzybkowska et al. 2020). In fact, they were common prey for perch and racer goby in our study.

Amphipods were especially important food items for perch and racer goby in the Bug River. Depending on the site, these fish mainly ate *Pontogammarus robustoides* or *Dikerogammarus villosus* and less *D. haemobaphes*. Field observations have shown that all three species are rather eurytopic (Bącela and Konopacka 2005; Grabowski et al. 2007; Żytkowicz and Kobak 2008). Nevertheless, they show some species-specific habitat preferences. For example, *D. villosus* and *D. haemobaphes* were reported to prefer stony substrates (Boets et al. 2010; Clinton et al. 2018). On the other hand, in comparison to adult individuals, juveniles of *P. robustoides* are known to prefer various macrophytes as their main habitat (Czarnecka et al. 2010). The high contribution of *P. robustoides* to the diet of the racer goby and the co-occurring European perch has also been reported in our earlier studies in the Włocławski Reservoir (Grabowska and Grabowski 2005). Amphipoda are known to be eaten by racer goby in the main channel of the Vistula River and in the large dam-reservoir built on it (Kakareko et al. 2005), as well as in its native range, i.e. in the middle Dnieper River (Pinchuk et al. 2003). Considering chironomid larvae, both perch and racer goby predated relatively large species, such as *Glyptotendipens* cf. *pallens*. This morphotype of *Glyptotendipes* spp. is common in various freshwater habitats (Moller Pillot 2009). Often, it is associated with macrophytes and coarse organic matter (Kornijów 1997; Moller Pillot 2009; Čerba et al. 2022). *Glyptotendipes pallens* is a plant tissues miner and scraper (Koperski 1998; Beiger 2004), but it also inhabits other types of substrates, for example, plant detritus, wood debris and mud (Moller Pillot 2009; Čerba et al. 2022). Macrophytes are traps for organic matter in running waters and create ideal microhabitats for bottom dwelling chironomids, thus, many *Glyptodendipes* spp. are common on macrophytes, as well as in mud gathered around them (Grzybkowska et al. 2020). *Glyptotendipes* sp. were also one of the most important Chironomidae taxa in the diet of racer goby in lowland rivers in the Dnieper River system (Didenko et al. 2021a) where, in line with our findings, the diet of racer goby also indicated its association with plants, as has been reported from the Vistula River (Kakareko et al. 2005). Epiphytic chironomids were found to be the main prey of perch in pondweed (*Potamogeton* spp.) patches, while typically benthic species were preferred by ruff (*Gymnocephalus cernuus*) in the lowland Warta River (Dukowska and Grzybkowska 2014). We did not record *Chironomus riparius* in the diet of perch at any site, while it was quite common in gut content of both goby species, however, with different contributions to the overall species diet. This sediment burrowing chironomid is probably more difficult to obtain by perch, contrary to both goby species, as they have a habit of hiding in sediments (Kakareko 2011), which may give them more opportunities to find *C.* cf. *riparius* larvae in mud. However, the European perch, considered to be an epi-benthic predator, was found to penetrate bottom sediments to some depth searching for food in lake littoral, where it fed on large individuals of *Chironomus plumosus* larvae (Kornijów 1997). Despite that, amongst the same lentic sedimentary benthos communities associated with littoral macrophytes, predation by perch was most intensive on motile invertebrates,

such as isopods and amphipods, while chironomids contributed less to perch diet (Kornijów et al. 2016). This is consistent with our results, suggesting that amphipods may be the most important prey for the European perch.

Both goby species and European perch feed on small gastropods, such as *Bithynia* sp., *Valvata* sp. and *Potammopyrgus antipodarum*, which are also associated with submerged macrophytes (Van den Berg et al. 1997). These gastropods were frequently recorded in the diet of the racer goby in the Włocławski Reservoir (Kakareko et al. 2005). Locally, they were even the dominant food category for that fish species (Kostrzewa and Grabowski 2003). In our study, in comparison with racer and monkey gobies, European perch rarely ate gastropods. Such prey was scarcely reported in previous studies on the diet of perch, even if they were abundant in the macroinvertebrate assemblages (Rezsu and Specziár 2006; Kornijów et al. 2016). Macroinvertebrates of such low mobility are not attractive prey for sight-dependent diurnal predators like perch (Craig 2008; Kornijów et al. 2016). Another food item that differentiated gobies and perch in terms of diet were the Sphaeridae bivalves. That typical benthic group of molluscs was found quite frequently in the diet of both racer and monkey gobies, while none was recorded from perch. Sphaeridae were also an important prey of both goby species in the Vistula River and in the Włocławski Reservoir (Kakareko et al. 2005). Coleopteran larvae were occasional prey of gobies and perch in the Bug River. In the gut content of racer goby, we even found an adult of Gyrinidae. The presence of pleuston organisms, such as whirligig beetles, suggests that racer goby utilised a wider range of microhabitats when searching for food, from the surface of the water to the riverbed. In fact, the diversity of the gobies' diets, especially in the case of racer gobies, was higher than in the case of perch.

In summary, alien gobies, in particular the racer goby, and European perch possibly used similar habitats for foraging, i.e. macrophyte patches in areas of more stagnant water and muddy bottom. However, perch with a body length similar to that of co-occurring gobies, was more piscivorous. The dietary overlap between perch and gobies usually comprised prey items that are very common in the riverine environment, such as amphipods and large chironomid larvae (Dukowska et al. 2012; Dukowska and Grzybkowska 2014). The two fish display different foraging strategies. Perch searches actively for prey, is a sight-dependent diurnal predator (Craig 2008) and prefers rather motile prey that are easier to detect (Kornijów et al. 2016). Activity of the prey seems to be less important for a nocturnal predator, such as the racer goby (Grabowska and Grabowski 2005; Kakareko et al. 2013). In experimental conditions, the racer goby fed equally effectively on immobilised and mobile amphipods, choosing prey species rather according to their quality than their mobility (Błońska et al. 2015), which suggests that, to detect food, the racer goby uses not only sight, but also other senses. Furthermore, perch is morphologically and anatomically better adjusted for active hunting and pursuing escaping prey than gobies that do not possess a swim bladder and have a less streamlined body shape.

Racer goby vs. monkey goby

Racer goby and monkey goby had similar diets. They fed mainly on Chironomidae larvae, on the basis of the relative abundance and frequency of this prey in the fish gut content. The detailed identification of taxa within this food category showed that, in fact, the gobies foraged in different microhabitats, even at the same sites and their mode of foraging was also slightly different. Our study shows that several

taxa of chironomids contributed to 60% dissimilarity between the diet of the studied goby species. Chironomids are a prevalent group in the freshwater macrozoobenthos, often standing out in their abundance and species and functional diversity, which makes them key elements of freshwater food webs (Armitage et al. 2012). Their ecological characteristics allow them to fill many niches and serve as a varied functional groups in aquatic ecosystems. Different groups of chironomid larvae are associated with different types of substrate: mud, sand, gravel, stones, plants (Moller Pillot 2009). They inhabit periphytic communities that develop on various hard surfaces or exploit the substrate by drilling into plant or animal tissue, mining wood, burrowing into the sediment surface or attaching to the bodies of other invertebrates (Moller Pillot 2009; Grzybkowska et al. 2016; Antczak-Orlewska et al. 2021). Chironomid larvae, being such a diverse group of macroinvertebrates and important food for many aquatic organisms, can be used as an additional indicator of habitat preferences, based on their contribution to the predator's diet.

Glyptotendipes cf. *pallens* dominated amongst chironomid larvae in the diet of racer goby. In summer, this taxon can be found in silty tubes built on macrophytes, mining in their decaying parts, but also on other firm surfaces, such as decaying wood or stones. In large rivers, in particular, the larvae of this species are more numerous on stones than on plants. This taxon avoids fast running waters and prefers more stagnant parts of the river channel (Moller Pillot 2009). Similarly, racer goby is more abundant in lentic areas, where it prefers habitats with a muddy bottom and moderate macrophyte cover, but also stones, for example, rip-raps along the river banks (Kakareko 2011; Płąchocki et al. 2020) or single stones scattered on the bottom (Kakareko et al. 2016). Thus, the high abundance of *G.* cf. *pallens* in the racer goby diet derives from similarity in habitats occupied by the prey and its predator. In addition to *Glyptotendipes* cf. *pallens*, the other chironomids associated with macrophytes (*Dicrotendipes nervosus* and *Polypedilum sordens*) were found in the gut content of the racer goby more frequently and in higher abundance than in the gut of the monkey goby. Similar chironomid taxa also dominated the racer goby diet in the Dnieper River system (Didenko et al. 2021a, b). Another indicator of racer goby habitat preferences are chironomids that use stones as one of the possible substrates, such as *Rheocricotopus chalybeatus*, which also frequently settles on plants and uses stones if plants are unavailable (Moller Pillot 2013). The *R.* cf. *chalybeatus* was recorded in the gut of racer goby more often than in monkey goby at two out of the three sites. Compared to racer goby and perch, the monkey goby ate many large larvae of the *Chironomus* cf. *riparius*. This pelophilous species is very common in chironomid communities associated with mud and sand, but sometimes also with submerged aquatic plants, burrowing in soft sediment trapped by the roots (Dukowska and Grzybkowska 2014; Grzybkowska et al. 2020; Leszczyńska et al. 2021). The species can be very numerous also on stones or concrete bottoms covered by a thin layer of mud (Moller Pillot 2009). *Chironomus riparius* is often considered to be a characteristic inhabitant of flowing waters, also fast-flowing sections of brooks and streams and even rapids, providing that the organic silt is on the bottom, as it feeds on organic particles. It was also recorded as the dominant chironomid taxon in the diet of monkey goby in the Vistula River and less numerous in the gut content of the co-occurring racer goby, which eats mainly epiphytic species (Kakareko et al. 2005). The monkey goby is usually associated with sandy or gravelly bottoms in lotic parts of rivers, while it is less abundant at sites with moderate vegetation cover (Kakareko 2011; Płąchocki et al. 2020). It also prefers higher

water velocity in comparison to the racer goby, as has been shown experimentally (Kakareko 2011) and can be found more often in the main flow of the river, where there are spots with slower water velocity caused by varied obstacles, such as macrophytes or stones. The latter form refuges for several organisms that are prey for fish, for example, for monkey goby. Such patches of macrophytes and stones covered by periphyton and accumulating sediment rich in organic matter, are very productive (Grzybkowska et al. 2020). Besides *C.* cf. *riparius*, other chironomid bottom-dwellers are more frequent in monkey goby gut content than in racer goby, for example, *Cladopelma* gr. *viridulum*, *Cryptochironomus*, *Lipiniella moderata*, *Microtendipes* cf. *pedellus*, *Stictochironomus* cf. *rosenschoeldi* (Brooks et al. 2007; Moller Pillot 2009). Some chironomid taxa recorded in the diet of monkey goby are typically associated with a fast water current and stony gravel substrate (e.g. *R.* cf. *chalybeatus*, *Rheotanytarsus* sp.) or with sand like, for example, *Lipiniella moderata* (Moller Pillot 2009; Klukowska et al. 2011). Moreover, the considerable contribution of caseless Trichoptera larvae, for example, *Hydropsyche* sp., which use water current to catch suspended organic matter, is another indicator that monkey goby occurs in lotic habitats (Stuijfzand et al. 1999). This supports the hypothesis of niche separation between the monkey goby and the racer goby and concurs with previous findings that monkey goby consumed mainly sand- and mud-dwelling, burrowing chironomids, while the racer goby has a more diverse diet, including both bottom-dwelling burrowing and phytophilous morphotypes of Chironomidae, as well as other macrophyte-associated macroinvertebrates (Kakareko et al. 2005; Didenko et al. 2022b). Shift in diel feeding activity can be another way to avoid food competition between co-occurring alien gobies. The racer goby is predominantly a nocturnal feeder (Grabowska and Grabowski 2005; Kakareko et al. 2013), while the monkey goby is more active during the day (Didenko et al. 2017) or shows no difference between day and night (Grabowska et al. 2009). Similarly, the co-existence of the other invasive gobies, i.e. round goby (*Neogobius melanostomus*) and big head goby (*Ponticola kessleri*) in the middle Danube River was suggested to be possible by resource partitioning and slightly different feeding strategy (Ševove and Kováč 2013). The previously published revisions of ecological interactions of five alien Ponto-Caspian gobies in their non-native range (Kornis et al. 2012; Grabowska et al. 2023) emphasised that they are a diverse group considering their ecological demands and functional ecology, including types of prey (e.g. Didenko et al. (2022b)) and diet shift with ontogeny (e.g. Ševove and Kováč (2016)); thus, their invasions in European inland waters impact native biota in diverse ways.

To conclude, we show that detailed prey identification to the lowest possible taxon is crucial to properly justify the diet overlap between co-occurring fish species and to verify the suggested impact of alien invaders on native species through interspecific competition. Resource partitioning considering prey types and foraging habitats is one of the ways of allowing the co-existence of closely-related alien gobies with similar food preferences in the invaded waters and their co-occurrence with local fish species. Together with an opportunistic feeding strategy, it is likely to be a major factor behind their invasion success observed in European waters in the last decades. We therefore recommend that, in order to gain more detailed insights into the foraging strategy of fish, in future studies, researchers should not limit their dietary analysis only to the identification of higher taxa, but should identify prey down to the lowest possible level, especially in taxonomic groups consisting of species that differ in the microhabitats they occupy.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

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Author ORCIDs

Joanna Grabowska https://orcid.org/0000-0001-9924-0650
Mateusz Płóciennik https://orcid.org/0000-0003-1487-6698
Michał Grabowski https://orcid.org/0000-0002-4551-3454

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Relative abundance of species (%N) in fish assemblages found at sites Z, R, B in the Western Bug River in August 2007 (Penczak et al. 2010)

Authors: Joanna Grabowska, Mateusz Płóciennik, Michał Grabowski

Data type: xlsx

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Supplementary material 2

Relative abundance of prey categories (%N) (number of given prey category in relation to total number of prey) identified in fish guts at sites Z, R, B in the Western Bug River in August 2007

Authors: Joanna Grabowska, Mateusz Płóciennik, Michał Grabowski

Data type: xlsx

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